Supplementary Material to:
Mulligan CJ, D'Errico NC, Stees J, Hughes DA. Methylation changes at NR3C1 in newborns associate with maternal prenatal stress exposure and newborn birth weight. Epigenetics 2012; 7(8); http://dx.doi.org/10.4161/epi.21180
http://www.landesbioscience.com/journals/epigenetics/article/21180

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Supplementary Materials and Methods:

Subjects and samples. Whole blood samples were collected from 25 women who delivered their babies at HEAL Africa hospital in Goma, eastern Democratic Republic of Congo in July-August, 2010. Umbilical cord blood samples were collected from discarded placentas from the same 25 women within several hours of delivery. Samples were collected with informed consent under Western IRB approval, Olympia, WA (WIRB Project # 20100993).

Ethnographic interviews and trauma surveys. Trauma surveys were adapted and expanded for cultural relevance from the Peritraumatic Distress Inventory (1). Ethnographic interviews (2) were used with each participant to cover reproductive history, past traumatic exposures, and general health history. Nearly all relevant information was obtained during interviews, but surveys provided standardized data since no two ethnographic interviews were identical although they covered much of the same thematic ground.

Sodium bisulfite DNA sequencing. Genomic DNA was extracted from 50 whole blood and umbilical cord blood samples using Qiagen QIAamp DNA Midi Kits according to the manufacturer's instructions and eluted in two separate volumes of 200ul (Qiagen, Valencia, CA). For each sample, 250ng DNA was treated with sodium bisulfite according to manufacturer's instructions (EZ DNA Methylation Kit, Zymo Research, Irvine, CA). A 321 bp region of the *NR3C1* promoter was amplified in two rounds of polymerase chain reaction (PCR) amplifications of 25ul each using previously published primers and conditions (3). PCR amplicons were electrophoresed on 1% agarose gels and DNA was extracted from excised bands using a 30ul elution volume with a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). DNA

fragments were cloned into a pGEM-T vector (Promega, Madison, WI) in a 5ul reaction with 0.5ul eluted DNA and incubated at 4°C overnight. Ligation reactions were transformed into DH10B cells, colonies were grown overnight and sequenced directly with the M13R primer on a Applied Biosystems Model 3130 at UF's Interdisciplinary Center for Biotechnology Research. An average of 21 colonies per sample was sequenced.

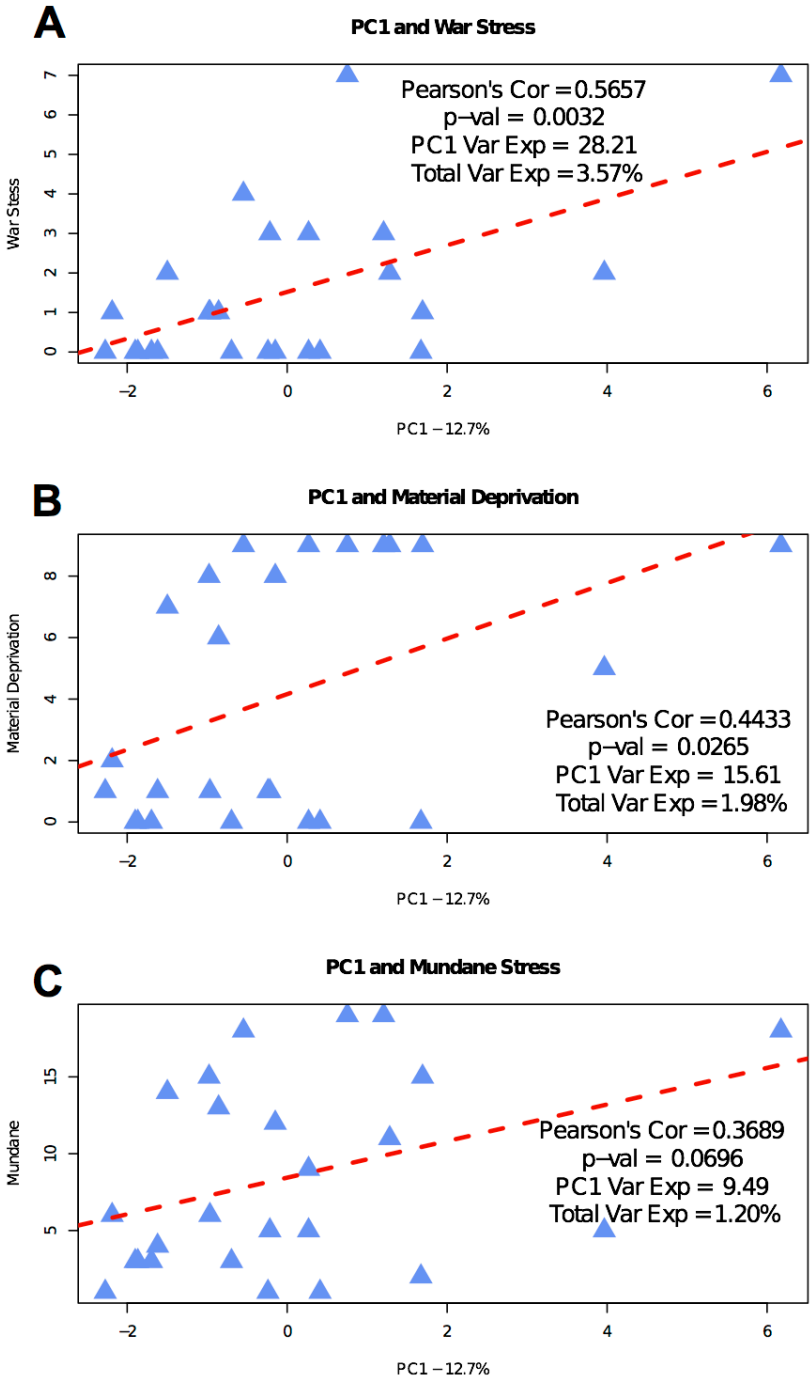
Statistical analyses. All statistical analyses including regressions, analyses of variance (ANOVAs) and principle component analyses (PCAs) were completed in R (4). Specifically, linear regression were carried out with lm(), regression correlations with cor.test(), ANOVAs with anova(), PCAs with prcomp() and factor analyses with the factanal() function - all from the stats package distributed by the R Project. Proportions of variances were estimated by calculating expected mean squares (EMS) through ANOVAs and identification of methyl markers driving particular principle components were identified through exploratory factor analyses.

References:

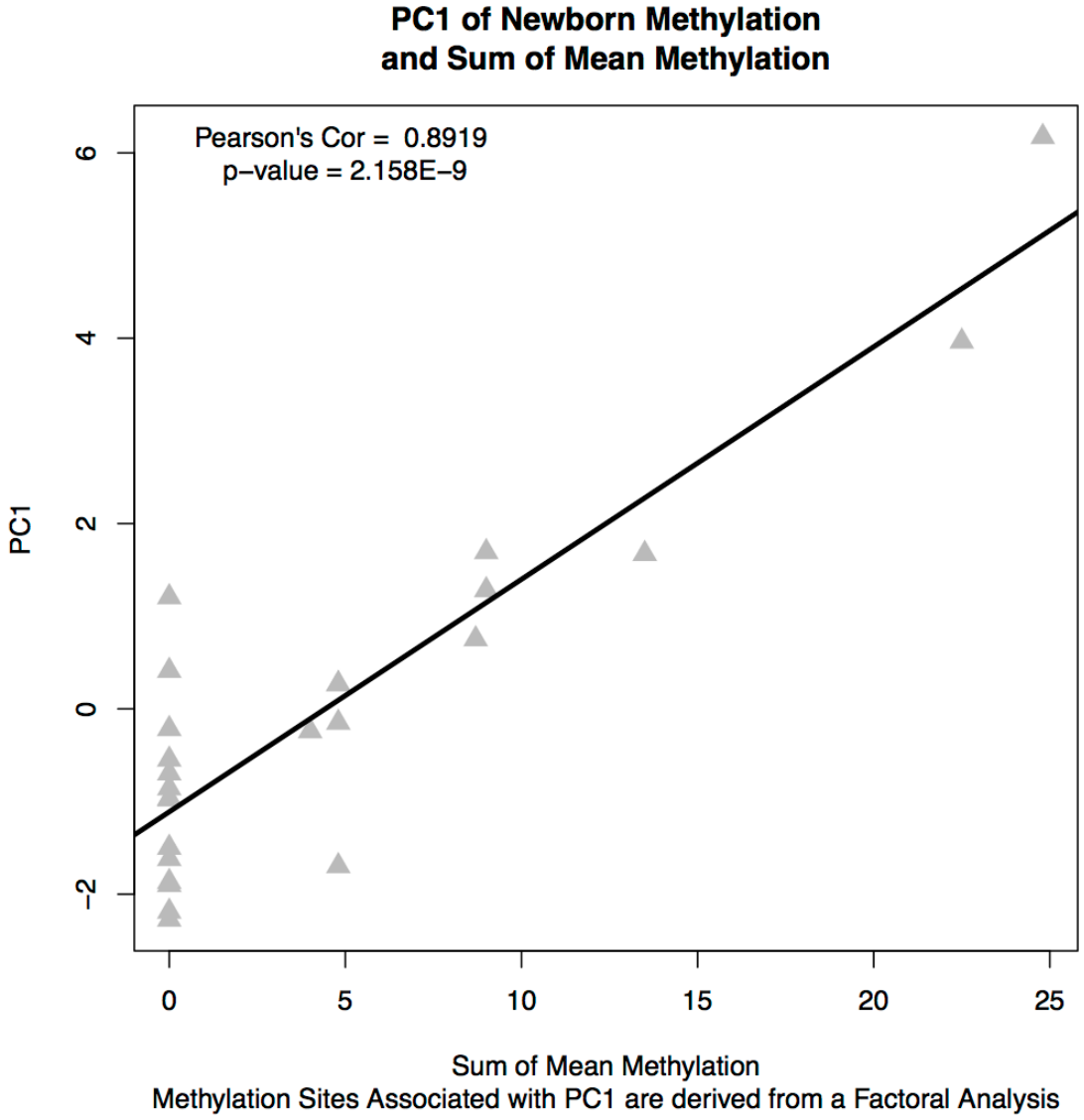
1. Brunet, A., Weiss, D. S., Metzler, T. J., Best, S. R., Neylan, T. C., Rogers, C., Fagan, J. & Marmar, C. R. 2001. The Peritraumatic Distress Inventory: A Proposed Measure of PTSD Criterion A2. *American Journal of Psychiatry* **158**, 1480-1485.
2. J. Spradley. 1979. *The Ethnographic Interview*. (Wadsworth, Belmont).
3. P. O. McGowan *et al.*, McGowan, P. O., Sasaki, A., D'Alessio, A. C., Dymov, S., Labonte, B., Szyf, M., Turecki, G., Meaney, M. J. 2009.Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* **12**, 342-348.

4. R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing. http://www.R-project.org (Vienna, Austria).

Supplementary Figure 1. Correlation of newborn methylation with maternal stressors. Newborn methylation was analyzed in a principal components analysis (PCA) and correlation of the first principal component (PC1) with three maternal stressors is shown here. Plot abbreviations are as follows: PC1 Var Exp = amount of variance in PC1 explained by war stress; Total Var Exp = total amount of methylation variance explained by war stress.



Supplementary Figure 2. Correlation between newborn methylation principal component 1 (PC1) and sum of mean infant methylation (sum of methylation percentages at the six sites driving PC1, i.e. M25, M20, M23, M10, M21, and M24 in order of decreasing loadings)



Supplementary Table 1. Maternal stress exposures and associated questions

Stressor	Questions
Material deprivation	Do you (or your husband) own your own home? Are you (or your husband) building your own home? Do you (and your husband) have a private room in your home? Did you have a wedding? Do you have clothes for your infant? Do you have new clothes for yourself? Is your purse in good condition? Is your hair braided? Are you wearing nail polish? How did you travel to the hospital?
Mundane stressors	Did you receive prenatal care? Did you choose the location where you gave birth? Did anyone travel with you to the hospital? Will you pay your hospital bill on time? Did your husband cook/clean during your pregnancy? Did you cry during your pregnancy? Were you stressed during your pregnancy? Were you beaten during your pregnancy? Were you ill during your pregnancy? Was this pregnancy wanted? Do you have a choice in reproductive decisions? Did you have enough to eat during your pregnancy? Have you had enough to eat in the past? Do you have help at home? Do you have a happy marriage? Does your husband have co-wives? Are you ashamed to cry? Do you have a chronic illness? Do you have stress because of your in-laws?
War stressors	Is your current pregnancy the result of rape? Have you been raped during your current pregnancy? Have you been raped in the past? Have you had a child as a result of a past rape? Are you/have you been a refugee? Has a family member been killed in the war? Have you ever been kidnapped? Was your birth the result of a rape? Are either of your parents the result of a rape?

Supplementary Table 2. Pearson's correlations across maternal stress models. Pearson's rho is reported in the lower half of the matrix and correlation p-values are reported in the upper half of the matrix.

	war	matdep	mundane
war		0.0003368287	0.0001548883
matdep	0.6593746		0.0000000758422
mundane	0.6856828	0.8499388	